AuSaMicS™ Life Science

TECHNICAL DATA SHEET

TDS-AS-1427-TBX
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Product Name	TBX Agar (Tryptone Bile X-glucuronide Agar)
Catalogue Number	AS-1427
LOT Number	TBX2601-01
HS Code	3821.00.00
Standard	ISO 16649
Format	Dehydrated powder
Manufacturer	AuSaMicS Pty Ltd, Thomastown VIC 3074, Australia

1. Product Description

TBX Agar (Tryptone Bile X-glucuronide Agar) is a selective chromogenic dehydrated culture medium designed for the rapid detection and enumeration of Escherichia coli in water, food, and environmental samples. The medium operates via enzymatic beta-glucuronidase (GUD) activity, producing blue-green colonies upon hydrolysis of the X-glucuronide chromogenic substrate. TBX Agar is compliant with ISO 16649-2 for the horizontal enumeration of beta-glucuronidase-positive E. coli in food and animal feeding stuffs.

2. Composition (per litre of prepared medium)

Component	Function	Amount (g/L)
Peptones (Tryptone)	Nitrogen and nutrient source	20.0
Bile Salts No. 1	Gram-positive suppression (selective agent)	1.5
X-beta-D-glucuronide (chromogenic substrate)	Substrate for beta-glucuronidase — colour development	0.075
Agar	Solidifying agent	15.0
Suspend (reconstitution)	—	36.6 g/L
Final pH at 25°C	—	7.2 ± 0.2

3. Physicochemical Specifications

Parameter	Specification
Appearance (powder)	Beige to cream, free-flowing powder
Appearance (prepared)	Light amber, clear to slightly opalescent gel
pH (prepared, 25°C)	7.2 ± 0.2
Gel strength	Firm gel at 44°C incubation temperature
Moisture content	≤ 6%
Storage temperature	15–25°C, dry, protected from light and moisture
Key chromogenic substrate	5-bromo-4-chloro-3-indolyl-beta-D-glucuronide (X-glucuronide)
Target enzyme	beta-glucuronidase (GUD, EC 3.2.1.31)
Incubation temperature	44 ± 0.5°C

Parameter	Specification
Incubation time	18–24 hours

4. Mode of Action

TBX Agar operates through two complementary selective and chromogenic mechanisms:

1. Selective action: Bile salts (deoxycholate/cholate mixture) suppress growth of Gram-positive organisms by disrupting their cytoplasmic membranes. This selectively enriches Gram-negative enteric bacteria, primarily coliforms and E. coli.
2. Chromogenic detection: The substrate X-glucuronide is hydrolysed by beta-glucuronidase (encoded by the uidA gene), constitutively expressed by ~94–97% of E. coli strains at 44°C. The released indoxyl moiety spontaneously dimerises and oxidises to form an insoluble blue-green dye (5,5'-dibromo-4,4'-dichloro-indigo), which precipitates at the colony site, providing direct visual identification of E. coli without further confirmatory testing.

5. Quality Control Performance

Test Organism	ATCC Strain	Inoculum (CFU)	Expected Result	QC Result
Escherichia coli	**ATCC 25922**	**≤ 100**	**Blue-green colonies; ≥70% recovery**	**Conforms**
E. coli O157:H7	ATCC 35150	≤ 100	Colourless (GUD-negative)	Conforms
Klebsiella pneumoniae	ATCC 13883	≤ 100	Colourless or partial inhibition	Conforms
Enterococcus faecalis	ATCC 29212	≤ 100	Inhibited; no growth	Conforms
Staphylococcus aureus	ATCC 25923	≤ 100	Inhibited; no growth	Conforms

6. Preparation Instructions

3. Suspend 36.6 g of TBX Agar powder in 1 litre of distilled or deionised water.
4. Mix thoroughly to completely dissolve the medium, heating to boiling with regular agitation until fully dissolved.
5. Autoclave at 121°C for 15 minutes. Do not overheat.
6. Cool to 50–55°C and pour into sterile Petri dishes or tubes. Allow to solidify.
7. Incubate inoculated plates at 44 ± 0.5°C for 18–24 hours under aerobic conditions.
8. Read results: blue-green colonies indicate E. coli (GUD-positive); colourless colonies indicate non-E. coli coliforms.

7. Colony Appearance Guide

Organism	Colony Colour	Interpretation
E. coli (GUD+)	**Blue-green**	**Confirmed E. coli — beta-glucuronidase positive**
E. coli O157:H7 (GUD–)	Colourless or pale	GUD-negative E. coli — not detected by chromogenic reaction
Other coliforms	Colourless	Non-E. coli Gram-negative enteric organisms

Organism	Colony Colour	Interpretation
Gram-positive organisms	Absent / inhibited	Suppressed by bile salts

8. Literature References

9. Manafi, M., & Ossmer, R. (1993). Fluorocult TBX Agar: a new chromogenic medium for the rapid detection of E. coli in water. Zentralblatt fur Hygiene und Umweltmedizin, 194(4), 380–386.
10. ISO 16649-2:2001. Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli — Part 2. International Organisation for Standardisation, Geneva.
11. Frampton, E.W., & Restaino, L. (1993). Methods for E. coli identification in food, water and clinical samples based on beta-glucuronidase detection. Journal of Applied Bacteriology, 74(3), 223–233.
12. Tryland, I., & Fiksdal, L. (1998). Enzyme characteristics of beta-D-galactosidase and beta-D-glucuronidase-positive bacteria and their interference in rapid methods for detection of waterborne coliforms and E. coli. Applied and Environmental Microbiology, 64(4), 1018–1023.